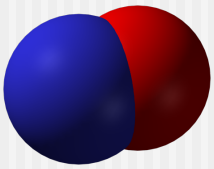
Nitric oxide

(·N=O)



vaclav.hampl@lf2.cuni.cz

http://physiology.cuni.cz

1

Why is NO interesting?

- Small anorg. molecule, large biol. importance
- Participates in the function of all main organ systems
- 2 faces: signalling x toxicity

- From the basic discovery to fundamental advances in clinical practice in a few years

4

History: 1620

NO first prepared:

Jan Baptista van Helmont

(Flemish, 1577-1644)

$Cu + HNO_3 \rightarrow Cu^{2+} + NO + H_2O$

(i.e. earlier than e.g. oxygen - 1774)

5

1772

Chemical characterization:

Joseph Priestley

(the discoverer of oxygen)

6

1800

Toxicity:

Sir Humphry Davy

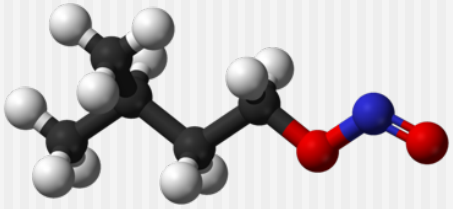
(almost died after inhaling NO)

7

1867

Amylnitrite ($C_5H_{11}ONO$) lowers blood pressure in hypertension

(today we know that this is due to NO release)



9

10

1977

NO activates guanylate cyclase, thus increasing intracellular cGMP concentration:

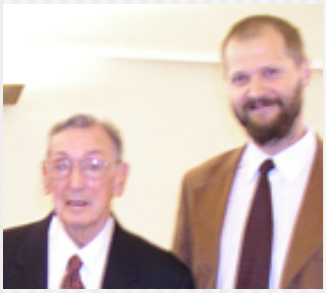
Ferid Murad

11

1980

Endothelium-derived relaxing factor (EDRF):

Robert Furchgott

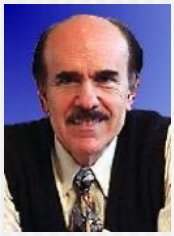


12

1987

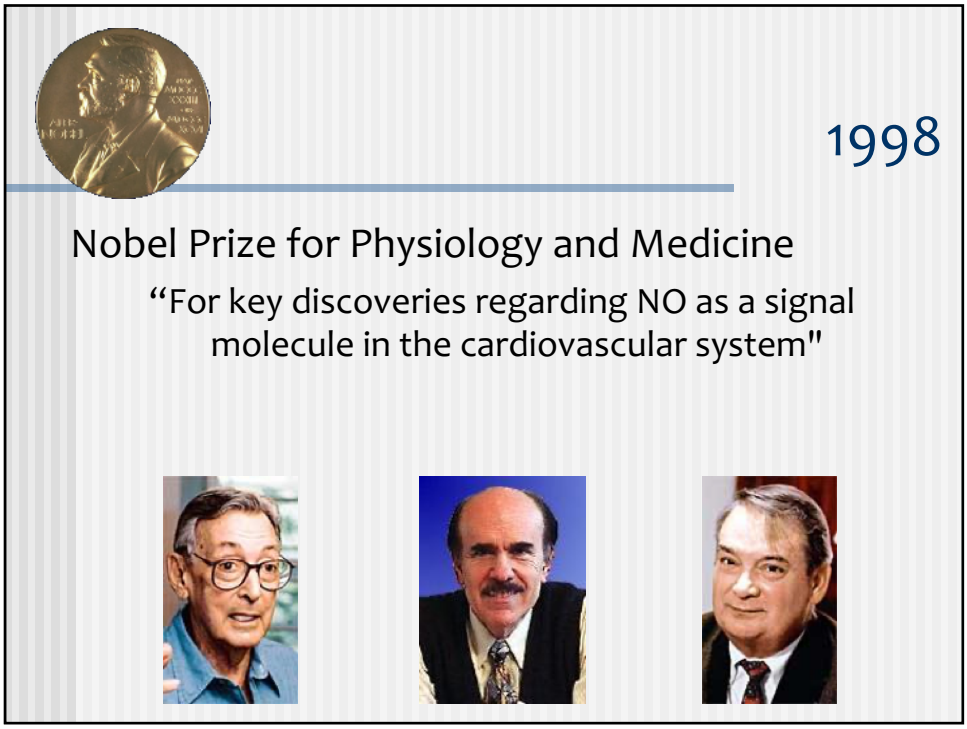
Eukaryotic cells can make NO:

Louis Ignarro, Salvador Moncada



13

14


1998
Nobel Prize for Physiology and Medicine
"For key discoveries regarding NO as a signal molecule in the cardiovascular system"

15

NO chemistry

- NO is a gas (colorless)
  (liquidifies at -152oC, solidifies at -164°C)
- NO is a radical
  - i.e. odd number of valency electrons
  - NO has 11 (N_2 has 10; O_2 has 12)
- Direct synthesis from N2 and O2 only under specific conditions (e.g. lightning)
- Also combustion engines, power plants

16

NO solubility

- Low solubility in water
  - ~1.7 mmol/l at 25°C
  - i.e. similar to O_2 or N_2
- Lipophility → easy passage through membranes

17

Spontaneous decay of NO

- Only under high pressure
- Gradual conversion to toxic NO_2

  (! storage in pressurized cylinders !)

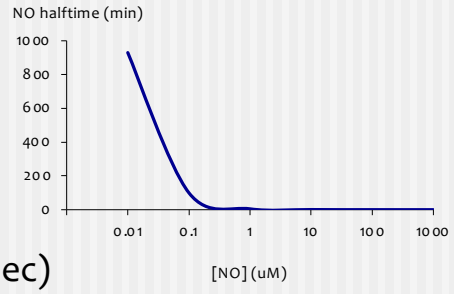
18

Oxidation of NO

- In the presence of O_2:

  $$2\ NO + O_2 \rightarrow 2\ NO_2$$

- NO_2 is a toxic radical (brown gas)
- Fast (several sec), if there is lots of NO and O_2
- Slow, if NO is scarce
  - that is usually the case in tissues (NO < 10 µM, NO half time ~ 500 sec)



19

Oxidation of NO

- ~ 200x faster in solution than in gas phase
- End products in solution: nitrites (NO_2^-), resp. HNO_2
- Proceeds to nitrates (NO_3^-) only in the presence of hemoproteins

20

Physiological role of NO_2^-

- "Storage" of NO in blood and tissues
- Easy reduction to bioactive NO
  - non-enzymaticaly
  - XORs, NOS, cytochromes, deoxyhemoglobin, deoxymyoglobin
- ↑NO_2^- reduction to NO at low O2 (helps hypoxic vasodilation)

21

Nitrates (NO_3^-) in food

- rich in leafy green vegetables (and some roots)
- reduced to NO_2^- by commensal bacteria on tongue
- NO_2^- further reduced to NO in the stochamch by low pH → kills almost all bacteria swallowed with food
- similarly protection of skin from fungi: NO_3^- in sweat reduced to NO_2^- by commensal microorganisms on skin and further to NO by the slightly acidic skin surface
- NO_3^- contribute to + effects of vegetables (prevention of cardiovasc. diseases and DM type 2)

22

Reaction of NO with superoxide

- O_2^- is a reactive oxygen radical
  - some is formed in respiratory chain
  - high production at inflammation sites (NADPH oxidase)
- O_2^- & NO form very quickly peroxynitrite:

$$NO + O_2^- \rightarrow OONO^-$$

- $OONO^-$ is not a radical, but is highly reactive (> O_2^-) & cytotoxic (also nitrosylates) and survives longer in biol. systems

23

Inactivation of NO by hemoglobin

- NO has a high affinity to heme
- Fast inactivation of NO by oxidation with Fe of oxyhemoglobin yielding NO_3^-

nitrosoHb --> metHb -->
Hb reductase --> oxyHb

24

S-nitrosylation of proteins

- reversible binding of NO groups to sulphydryl groups of proteins (posttranslational modification)
- affects
  - receptors coupled with G proteins
  - mitochondrial metabolism
  - $[Ca^{2+}]_i$
  - cell defense against oxidative stress & apoptosis

25

Measuring NO

- Chemiluminescence ($NO + O_3 \rightarrow NO_2^* + O_2 \rightarrow NO_2 + h\nu$)
  - gas phase
  - liquid phase (stripping)
  - NO oxidation products (reducing chamber)
- Elektroanalysis (amperometry) - NO reacts with electrode → Δ current of voltage
- Spin trap: NO + Fe-dithiocarbamate complexes, then detection of mono-nitrosyl-Fe complexes by electron paramagnetic resonance (EPR)
- Fluorescence indicators (4,5-diaminofluorescein - DAF-2): intracellular measuremens

26

Measuring NO

27

NO biosynthesis

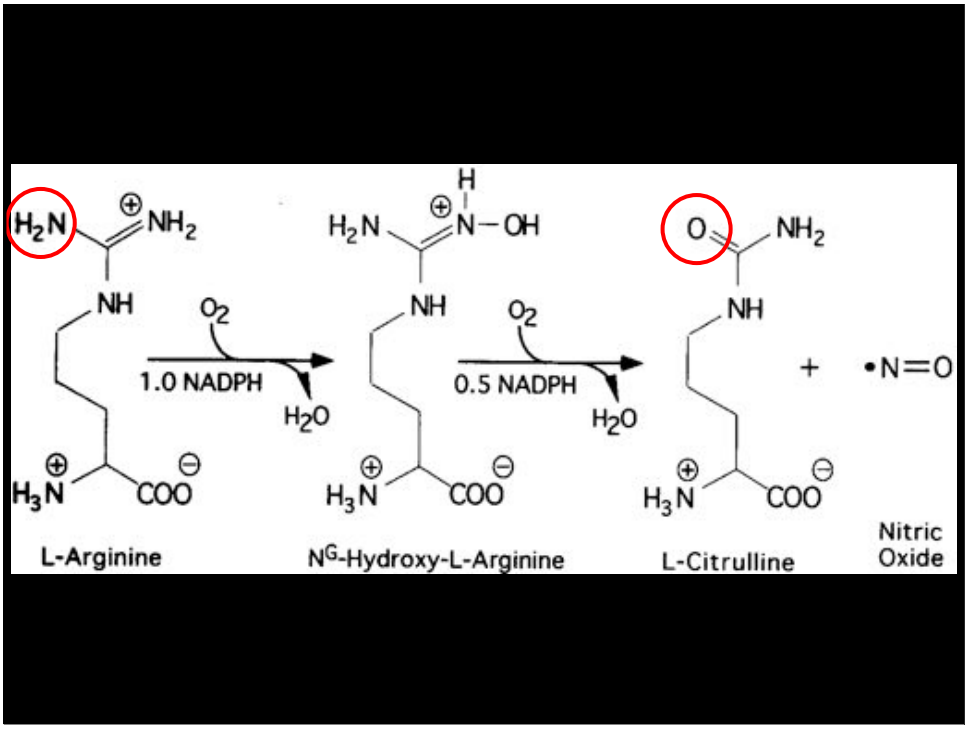
- 5 electron oxidation of terminal guanidino nitrogen of L-arginine by molecular oxygen :

  L-arg + O_2 --> NO + L-cit

- Stereospecificity

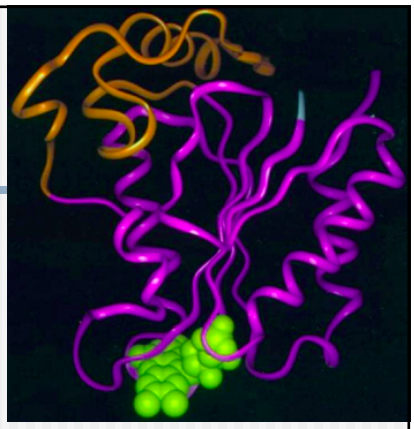
- The whole reaction is catalyzed by a single enzyme, **NO synthase** (NOS, EC 1.14.13.39)

28



29

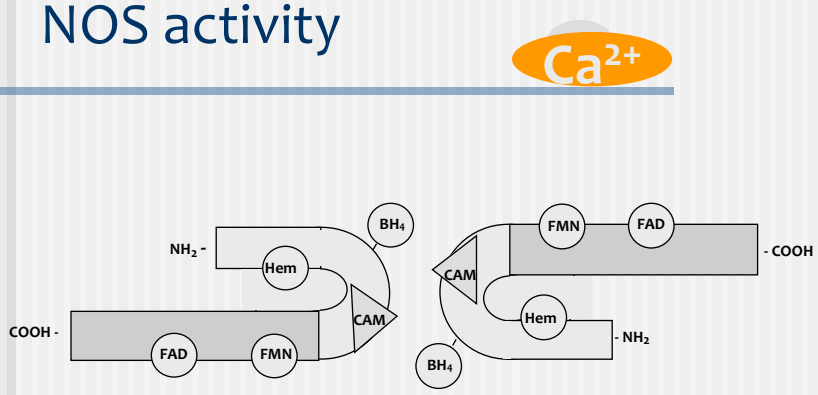
NO synthases



- 3 isoforms: I, II, III
  - all contain heme in the active center
  - active as homodimers
  - required cofactors:
    - NADPH
    - 6(R)-5,6,7,8-tetrahydrobiopterine
    - FAD
    - FMN
    - calmodulin

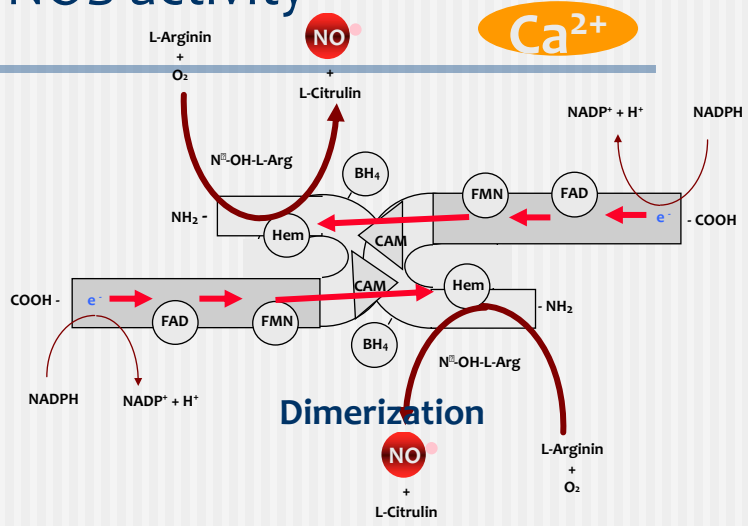
30

NOS activity



31

NOS activity

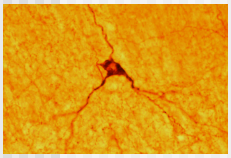


32

NOS uncoupling

- Uncoupling of the eNOS dimer (e.g. at↓BH_4, e.g. in stress) → consumption of NADPH and reduction of O_2 detached from NO production → ↓ NO profuction, ↑O_2^- production
  - one of the main mechanisms of endothelial dysfunction
- Vitamin C recycles BH_3 radical (created e.g. by $ONOO^-$) back to BH_4, also prevents BH_4 oxidation → acts against NOS uncoupling

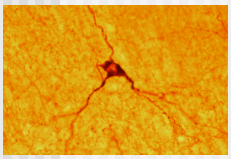
33



NOS I

- nNOS (neuronal)
- ~160 kDa
- Gene on human chromosome 12
- Requires Ca^{2+}
  (essential for calmodulin binding)
- Dissolved in cytosol

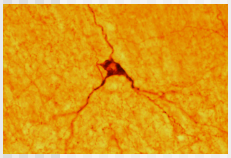
34



NOS I

- Constitutively expressed:
  - central and peripheral neurons
  - some epithelial and vascular smooth muscle cells
  - skeletal muscle
- Regulation of activity:
  - Ca^{2+}
  - ser/tyr phosphorylation
  - NO (feedback inhibition)

35

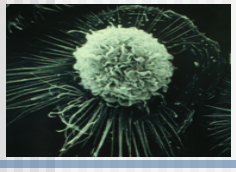


NOS I

Main function:

- neurotransmission
- neuromodulation

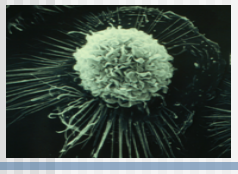
36



NOS II

- iNOS (inducible)
- ~130 kDa
- Gene on human chromosome 17
- Does not need Ca^{2+}
  (binds calmodulin permanently without Ca^{2+})
- Dissolved in cytosol

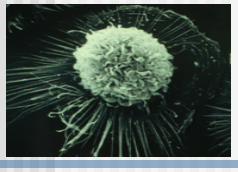
37



NOS II

- Expression is inducible (cytokines,...):
  - macrophages
  - glial cells, hepatocytes
  - endothelium, epithelium
  - cardiac myocytes, smooth muscle,...
- Regulation of activity:
  - induction of expression
  - NO (feedback inhibition)

38

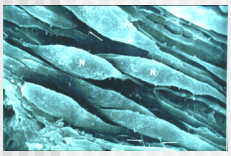


NOS II

Main function:

- fighting infection
- killing tumors

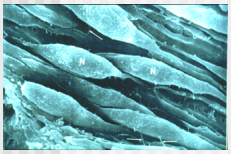
39



NOS III

- eNOS (endothelial)
- ~133 kDa
- Gene on human chromosome 7
- Requires Ca^{2+}
  (essential for calmodulin binding)
- Bound to cell mebrane (caveolae)

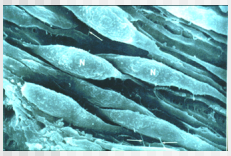
40



NOS III

- Constitutively expressed:
  - endothelium
  - pulmonary and renal epithelium; thrombocytes
  - cardiac myocytes
  - hippocampus
- Regulation of activity:
  - Ca^{2+}
  - ser/tyr phosphorylation
  - modulation of expression
  - inhibition by S-nitrosylation (thiol groups of cysteins)

41



NOS III

Main function:

- vascular tone regulation
- regulation of blood supply to organs

42

Mitochondrial NOS

- Similar to NOS I
- Importance unknown

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Effects of NO on the target tissues

1. Cytotoxicity:
   - at high NO concentrations (iNOS)
   - damage to proteins, DNA, lipids
   - oxidation (O_2, O_2^-)
     —> reactive, toxic products (NO_2, $ONOO^-$)
   - inhibition of respiration
   - fights infection and tumors

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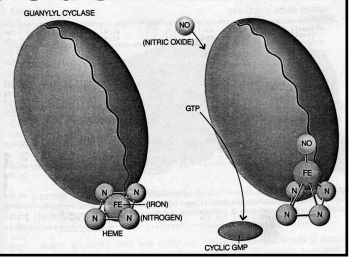
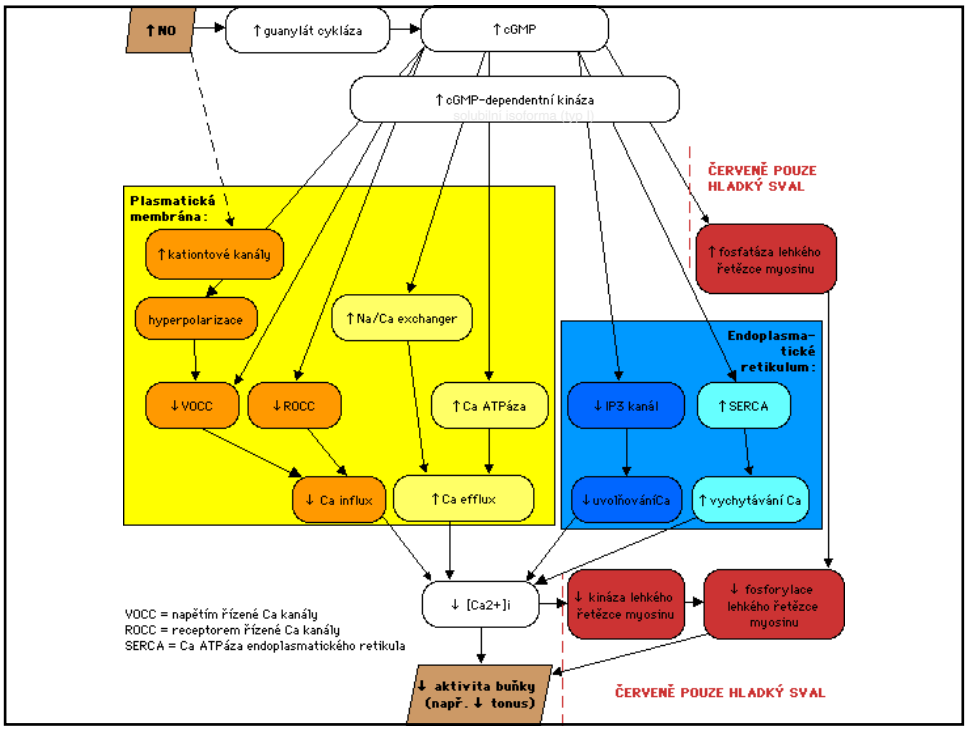
Effects of NO on the target tissues

2. Via cGMP:
   - At lower NO concentrations
     (eNOS, nNOS)
     Oxidation slow
   - Binding of NO to the heme of the soluble isoform of guanylate cyclase prevails
   - ↑ guanosine-3',5 ' monophosphate (cGMP)
   - cGMP activates cGMP-dependent protein kinase (G-kinase)



45


↑ NO
↑ guanylát cykláza
↑ cGMP
↑ cGMP-dependentní kináza
solubilní isoforma (typ I)
ČERVENĚ POUZE HLADKÝ SVAL
Plasmatická membrána:
↑ kationtové kanály
hyperpolarizace
↑ Na/Ca exchanger
↑ fosfatáza lehkého řetězce myosinu
Endoplasmatické retikulum:
↓ VOCC
↓ ROCC
↑ Ca ATPáza
↓ IP3 kanál
↑ SERCA
↓ Ca influx
↑ Ca efflux
↓ uvolňování Ca
↑ vychytávání Ca
↓ [Ca2+]i
↓ kináza lehkého řetězce myosinu
↓ fosforylace lehkého řetězce myosinu
VOCC = napětím řízené Ca kanály
ROCC = receptorem řízené Ca kanály
SERCA = Ca ATPáza endoplasmatického retikula
↓ aktivita buňky (např. ↓ tonus)
ČERVENĚ POUZE HLADKÝ SVAL

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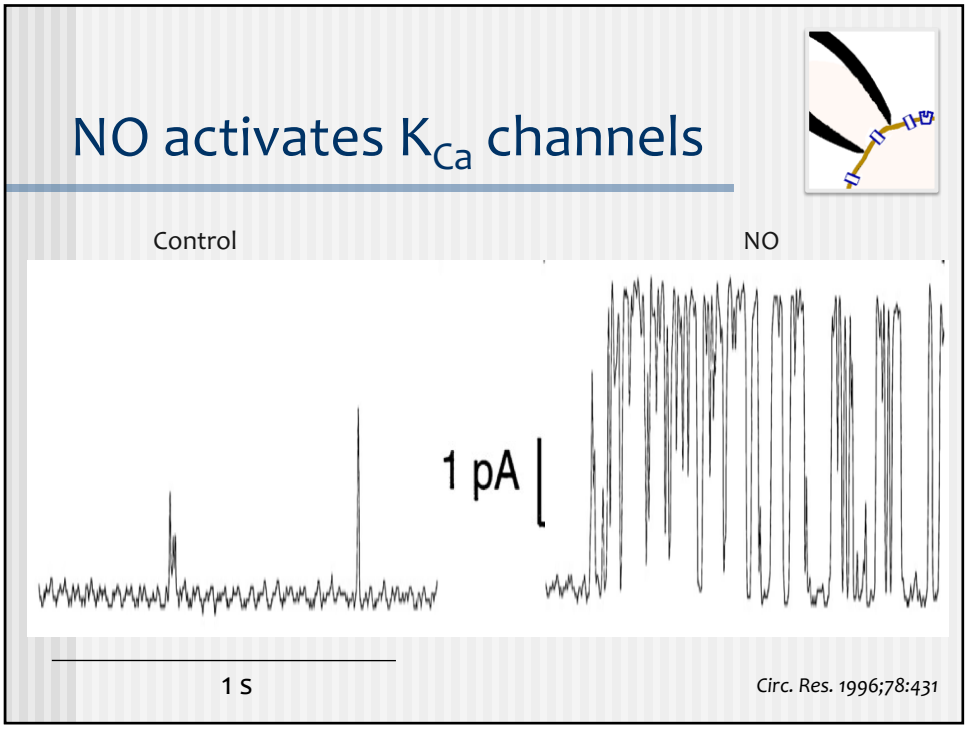

NO activates K_{Ca} channels
Control
NO
1 pA
1 s
Circ. Res. 1996;78:431

47

Fate of cGMP

cGMP inactivation:

Phosphodiesterases of cyclic nucleotides

- particularly type V.
- produce 5'-GMP

48

Pharmacology of NO

- NO donors
  (nitroglycerin, nitroprusside, NOates)
- NOS inhibitors
  (L-NMMA, L-NAME, aminoguadinine, 7-nitro-indazole, ADMA)
- eNOS activators
  (endothelium-dependent vasodilators)
- Inhibitory Phosphodiesterase inhibitors
  (Sildenafil, Zaprinast)

49

Functions of NO: Neurotransmission

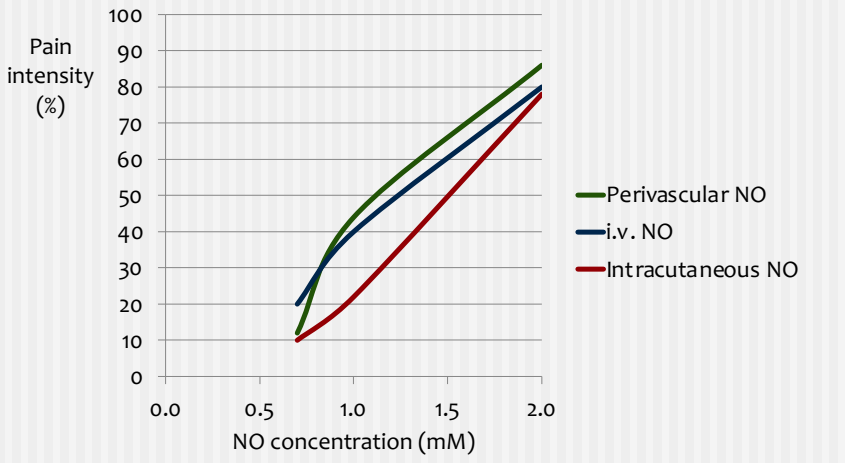
- Diffuse modulation
- NANC
- Retrograde messenger

  (confirms message receipt to the sender)
- Long-term potentiation

  (presynaptic cell programmed to next send a stronger signal - underlies memory)
- Learning, memory, sleep, pain, depression

50

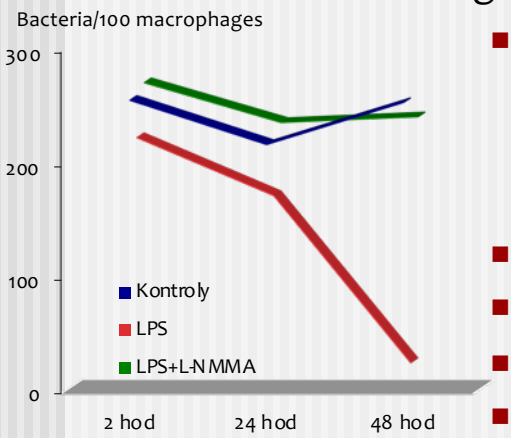
NO = mediator of peripheral nociception



Holthusen & Arndt: J Physiol 1995

51

Functions of NO: Cytotoxicity



Fights infection & tumors:

- bacteria
  (even those otherwise difficult to kill - e.g. Mycobacterium tuberculosis)
- fungi
- parasites
- tumors
- inhibits viral replication

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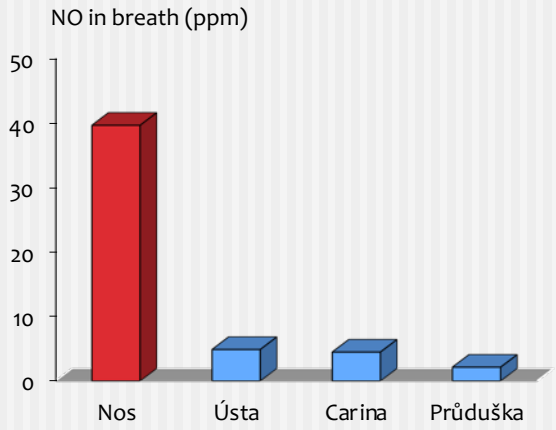
NO in breath

NOS most abundant in nose and paranasal cavities

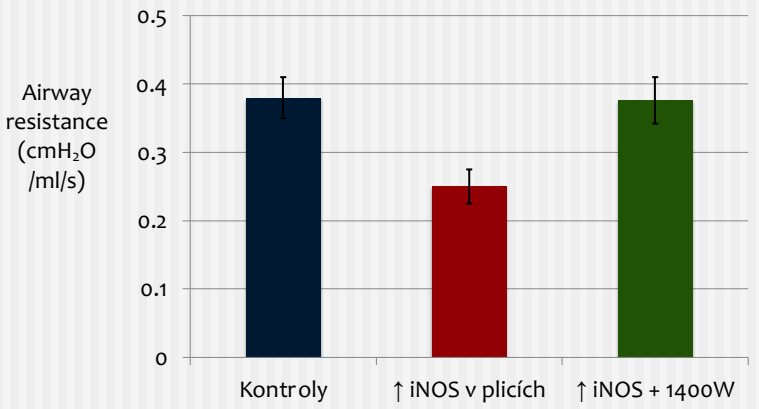
- Desinfection ?
- Bronchodilation
- Regulation of pulmonary vessels?
- Altered in some diseases (e.g. asthma)



Dillon et al, Chest 1996

53

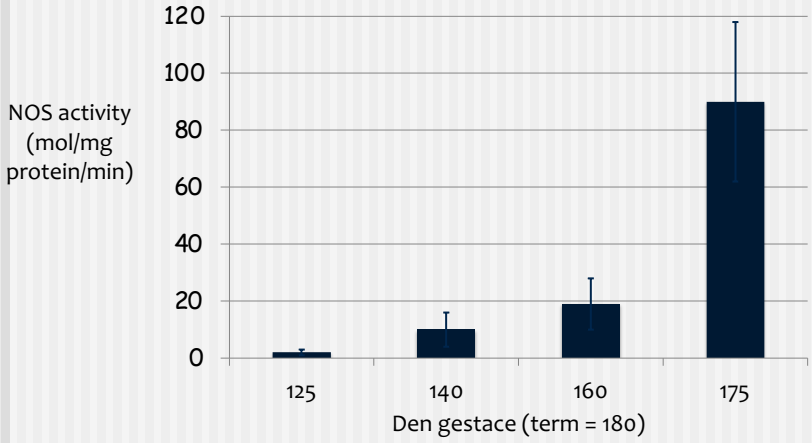
NO in airways reduces their resistance



Hjoberg et al: J Appl Physiol 2004

54

NO in airways rises before birth



Shaul: Am J Physiol Lung 2002

56

NO is not the only gaseous mediator

- Other gasotransmitters:
  - H_2S
  - CO

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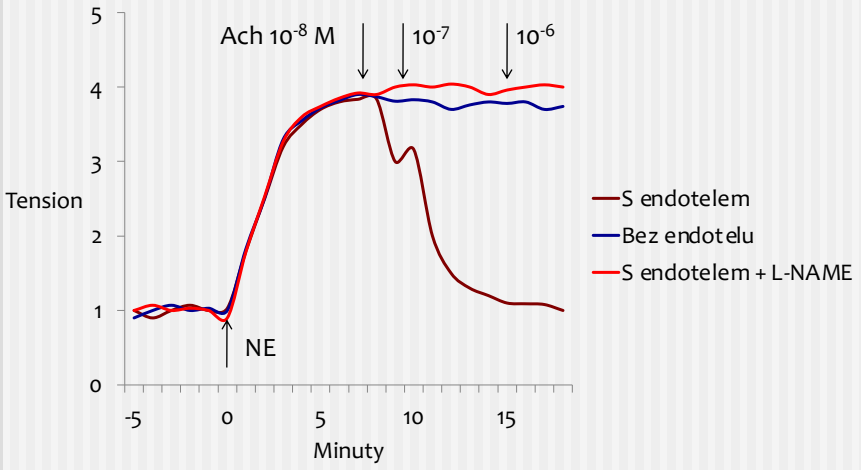
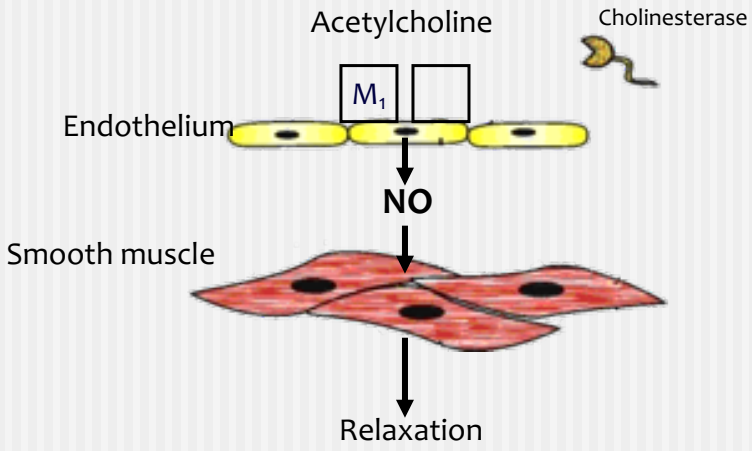
Endothelium-dependent vasodilation



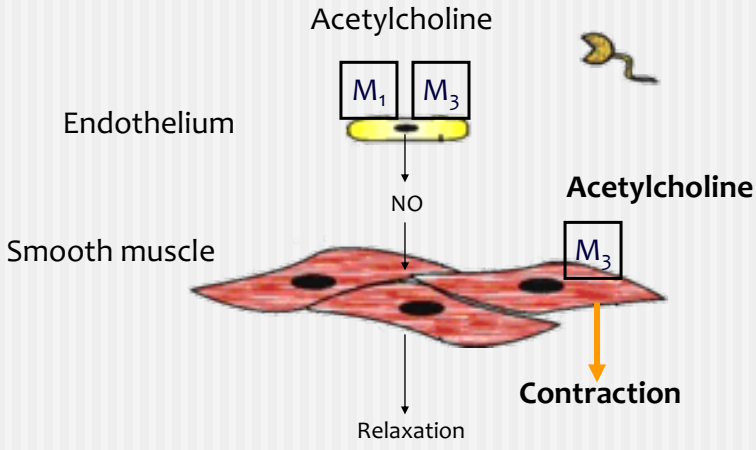
59

Endothelium-dependent vasodilation



60

Endothelium-dependent vasodilation



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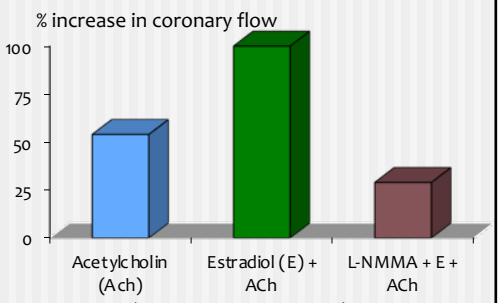
Endothelium-dependent vasodilation

Potentiated by:

- estrogens
  - premenopausal women ↓ risk of cardiovascular diseases
  - after menopause their risk = men
  - ↑ in pregnancy, esp. in uterus (x preeclampsia)
- insulin
  - ↑ glucose delivery to tissues (by ↑ blood flow)



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Functions of NO: regulation of blood vessels

Flow-induced vasodilation:

vasodilation in peripheral organs

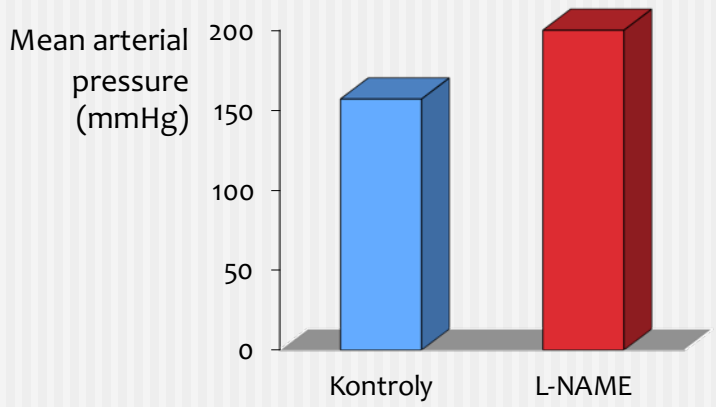
→ speed of blood flow in more proximal arteries
→ ↑ shear stress
→ ↑ eNOS activity (& expression)
→ vasodilation in proximal arteries

NO is indispensable in this function

(dysfunction causes hypertension)

63

„Tonic “ NO production



Isaacson: J Appl Physiol 1993

64

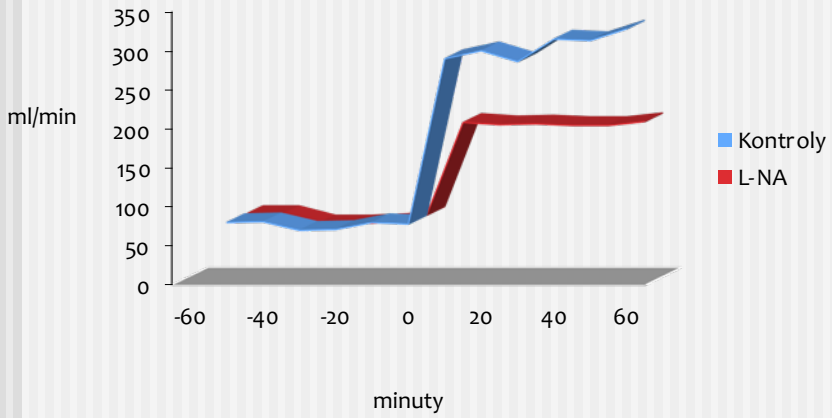
Endogenous NOS inhibitors

- Asymetric dimethylarginin (ADMA)
- N^G-monomethyl-L-arginine (L-NMMA)

↑ ADMA -> ↑ risk of atherosclerosis

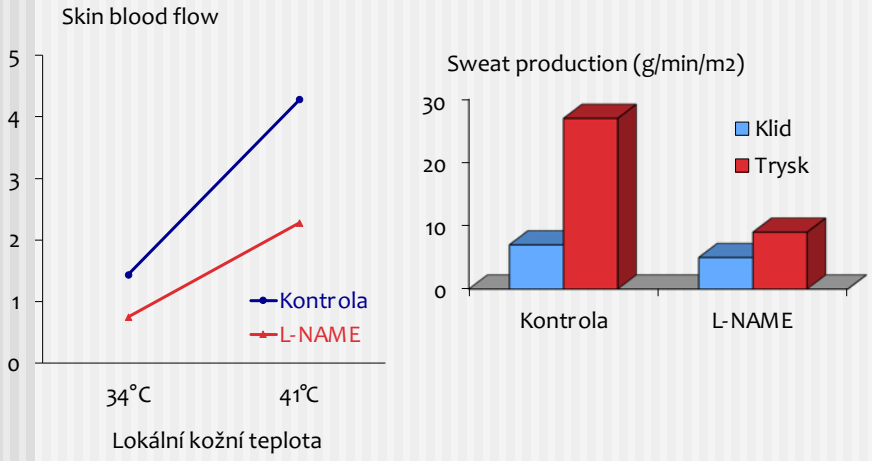
65

Pulmonary blood flow at birth



66

NO & thermoregulation

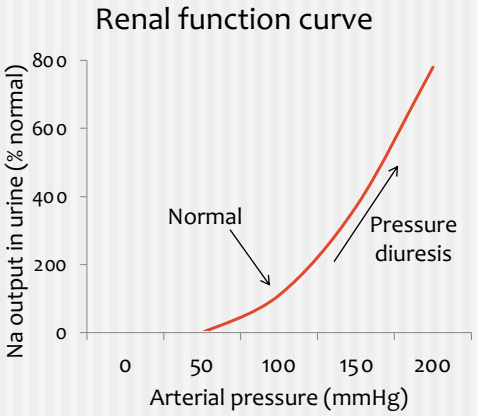


67

NO in kidneys

Mediates pressure diuresis:

↑ arterial pressure
→ mechanical strain of the endothelium
→ ↑ NO synthesis
→ diffusion to tubules
→ ↓ Na+ reabsorbtion



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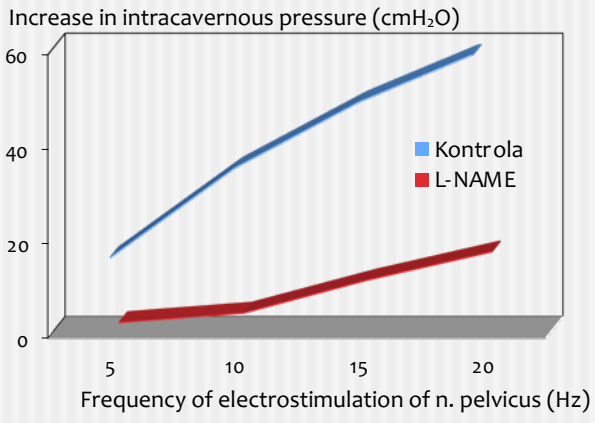
NO & penile erection

NO from n. pelvici terminals relaxes cavernous smooth muscle



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Funkce NO

- Snižuje srážlivost krve
  - Inhibice adhese, agregace a sekrece trombocytů
  - Aktivované trombocyty také tvoří NO - zpětnovazební inhibice agregace

    Fylogeneticky staré - krabi před 500 milióny let (hodně dlouho před savci)

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NO inhibuje apoptózu

- Apoptóza: "fysiologický" způsob smrti
- Na rozdíl od nekrózy nepůsobí zánět
- NO (→ cGMP → G kináza) inhibuje apoptotické fosforylační signály
- NO přímo inhibuje kaspázy

  (specifické proteázy apoptózy)

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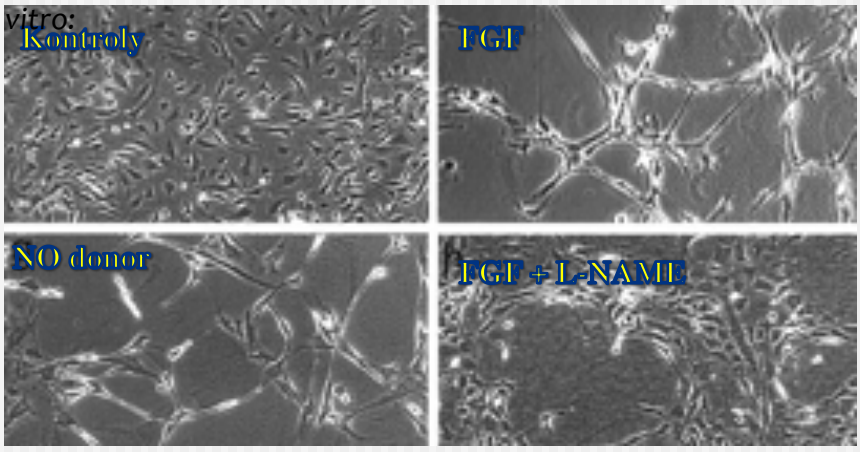
Dvojí role NO při ischémii a reperfúzi

- NO v malém množství chrání před ischemickým poškozením
- NO je důležité pro preconditioning
- ale NO přispívá k reperfuznímu poškození (excesivní NO tvořené během reperfuse reaguje s O_2^- → $ONOO^-$)

73

NO podporuje angiogenesi

Endoteliální buňky *in vitro*:



Saeid Babaei et al: Circ. Res. 1998

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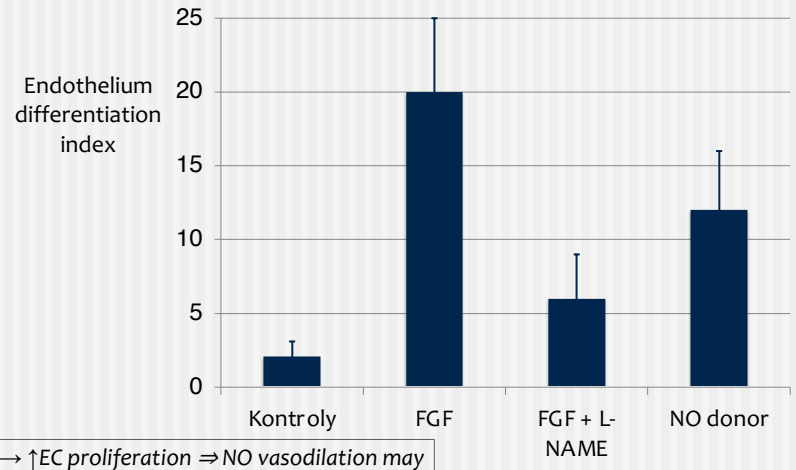
NO = mediátor angiogenese



↑flow → ↑EC proliferation ⇒ NO vasodilation may play a role in its angiogenic effects

Saeid Babaei et al: Circ. Res. 1998

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NO a dlouhověkost

eNOS- myši:

- předčasné stárnutí
- ↓dožití
- kalorická restrikce jim neprodlouží život

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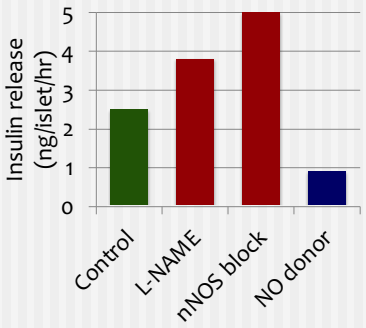
NO a sekrece insulinu

- glokóza stimuluje NOS v ostrůvcích
- tento NO inhibuje glukózou-stimulované uvolňování insulinu (negativí zpětná vazba)
- ↑aktivita NOS navozená hyperglykémií může přispívat k narušení glukózou stimulovaného uvolňování insulinu u diabetu 2. typu



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NO v kosterním svalu

- sval má všechny izoformy NOS (včetně pro sval specifické splice varianty nNOS)
- NO tlumí kontrakci (E-C coupling)
- NO ovlivňuje autoregulaci průtoku krve, respiraci a glukózovou homeostázu
- NO moduluje diferenciaci myocytů

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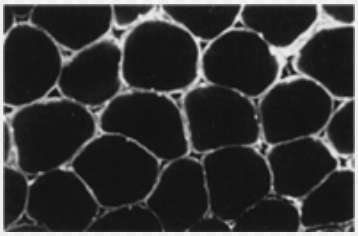
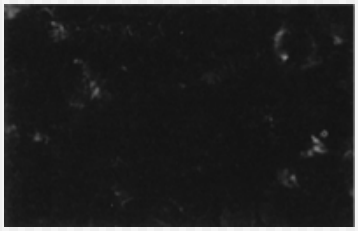
Absence nNOS ve svalu u Duchennovy muskulární dystrofie



zdraví



nemocní

Stamler & Meissne: Physiol Rev 2001

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Další funkce NO

- NO z endokardu moduluje **srdeční** kontraktilitu
- Esenciální negativní regulátor proliferace **při vývoji** (bez zastavení růstu není diferenciace)
- **Kost:**
  - Hodně NO (např. estrogenem, namáháním - NOS I):
    - inhibice resorpce (inhibicí tvorby a aktivity osteoklastů)
  - Málo NO:
    - potenciace resorpce indukované cytokiny
    - asi esenciální pro normální funkci osteoklastů
- Podíl na regulaci **laktace** (?)

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Patofysiologie NO

- septický šok
- hypertenze (?)
- atheroskleróza
- angina pectoris
- záněty, autoimunita
- erektilní dysfunkce
- diabetes mellitus (?)
- mozková mrtvice, roztroušená skleróza, Alzheimer (?), Parkinson (?)

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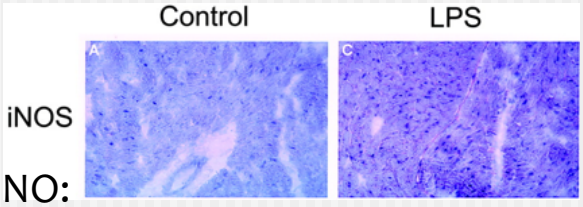
Septický šok

- Infekce (endotoxin) indukuje expresi iNOS



- Vysoká tvorba NO:
  - likvidace infekce
  - ale i vazodilatace → masivní hypotenze
  - indukce iNOS i v myokardu → ↓ kontraktilita

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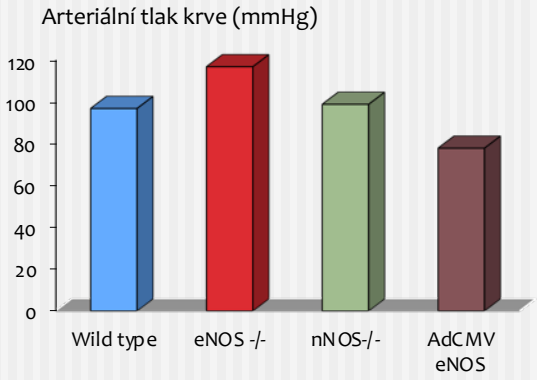
NO a hypertenze

- ↓ eNOS → hypertenze

Ale:

- syntéza NO nebývá při hypertenzi ↓ (někdy je ↑)



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NO a hypertenze

- NO v mozku inhibuje sympatickou aktivitu
  - lokální inhibice NOS v mozku

→ ↑ systémový tlak krve

(aktivace sympatiku se kriticky podílí na vzniku hypertenze)

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NO a hypertenze: Σ

- Dysfunkce NO není prvotní příčinou hypertenze
- Poškození endotelu vysokým tlakem ovšem může sekundárně narušit tvorbu NO
- To pak hypertenzi dále zhoršuje

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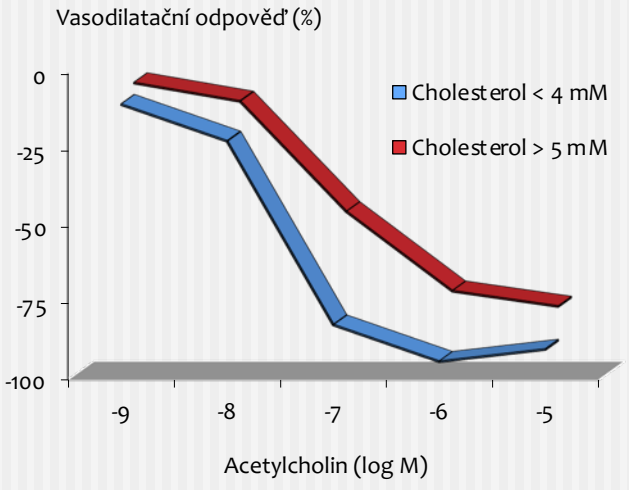
NO při hypercholesterolémii



Lidské koronární arterioly in vitro

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NO a atheroskleróza

Atherosklerotický plát
→ endoteliální dysfunkce
→ ↓ tvorba NO
→ paradoxní vazokonstrikce
(např. koronární cévy při námaze - angina pectoris)
→ ↓ ochrana proti tvorbě trombů
→ např. až infarkt

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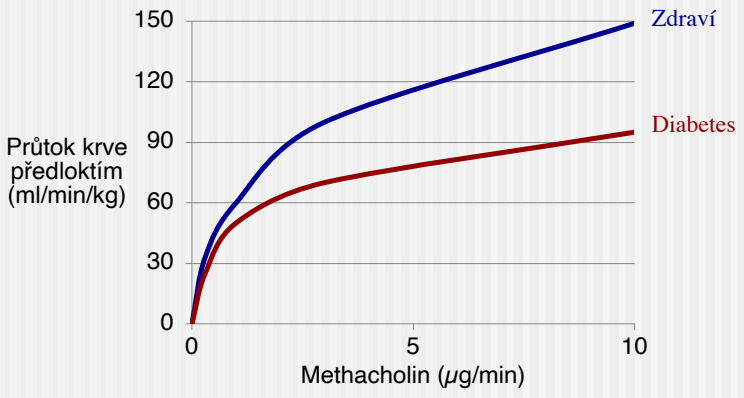
NO a diabetes

- porucha NO-dependentní vazodilatace



Circulation 88: 2510, 1993

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Patologie CNS
(např. mrtvice, roztroušená sklerosa,...)

- zánětlivá aktivace glie
- indukce iNOS
- NO inhibuje respiraci neuronů
- uvolnění glutamátu
- excitotoxická smrt

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NO v diagnostice

- Exhalovaný NO:
  - Záněty dýchacích cest
- NOx v plasmě
  - Polytrauma

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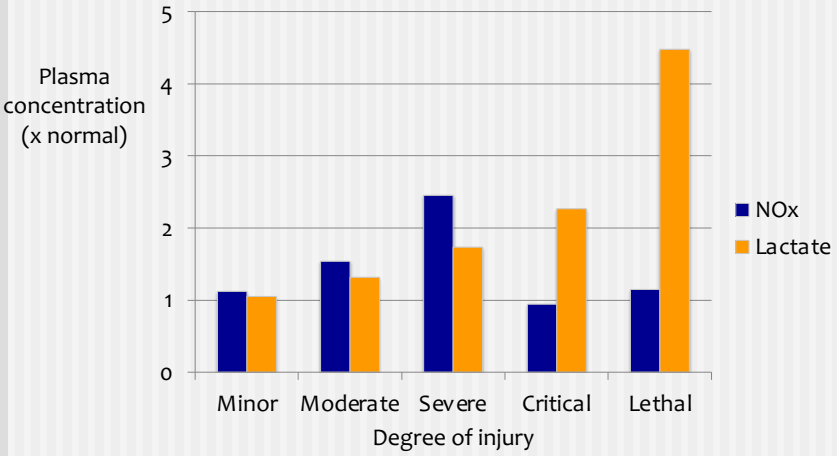
Serum NOx in polytrauma



Beitl et al: *Nitric oxide as an indicator for severity of injury in polytrauma, 2016*

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NO v terapii

- Inhalace plynného NO:
  - PPHN
  - ARDS
- Šok - pokusná terapie inhibitory NOS (aminoguanidin)
  (výsledky zatím nejednoznačné)
- Erektilní dysfunkce – Viagra (inhibitor PDE V.)
- NOáty

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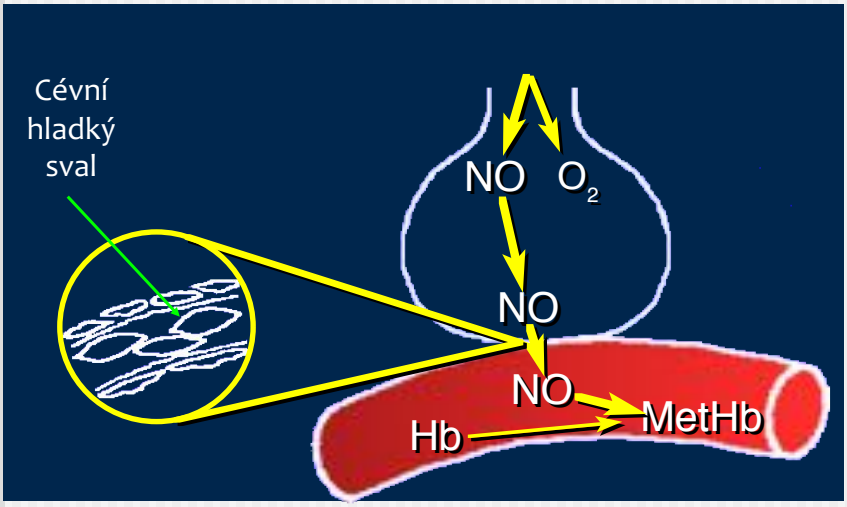
Selektivita inhalovaného NO pro plicní cirkulaci



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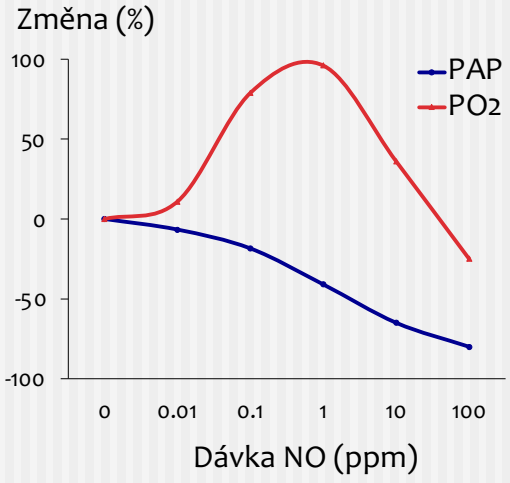
Inhalace NO při ARDS



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Inhalace NO při PPHN

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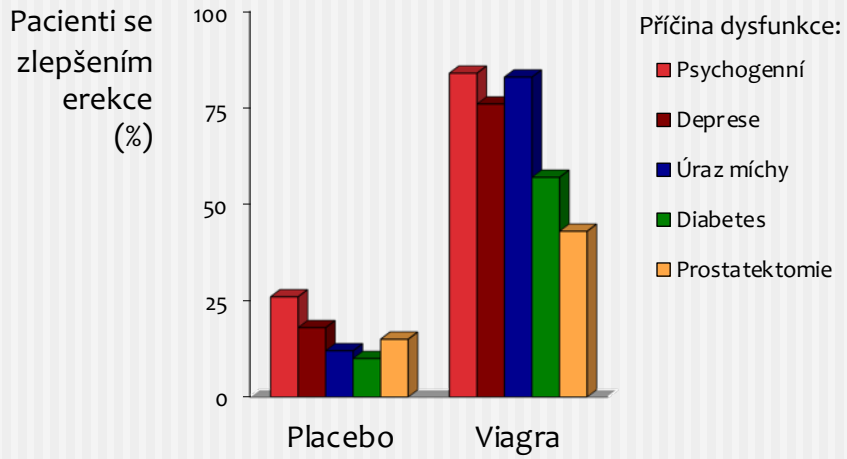
Sildenafil



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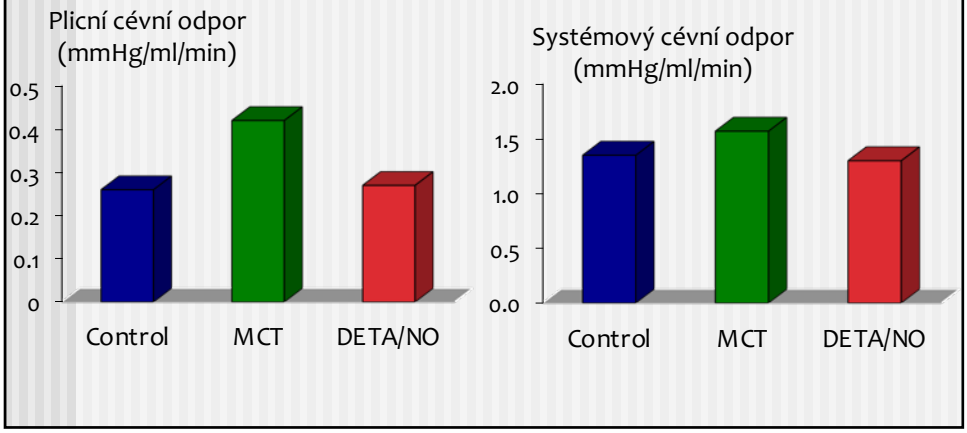
NOáty v terapii plicní hypertenze



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